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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/024,460	BERRY ET AL.	
	Examiner	Art Unit	
	Christian L. Fronda	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 November 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *61-80* is/are pending in the application.

4a) Of the above claim(s) *73-80* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *61-72* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *17 December 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *09/17/2002*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicants' election without traverse of Group I, claims 61-72, in the reply filed on 11/18/2004 is acknowledged. The requirement to elect a single protein in claim 70 has been withdrawn in view of applicants arguments filed 11/18/2004. Claims 73-80 withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention. The requirement is still deemed proper and is therefore made FINAL.

2. Claims 61-72 are under consideration in this Office Action.

3. The paper copy and computer readable form (CRF) of the Sequence Listing filed on 12/17/2001 have been received and have been processed by the Scientific and Technical Information Center (STIC).

Claim Rejections - 35 U.S.C. § 112, 2nd Paragraph

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 61-72 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 61 is vague and indefinite because while the preamble recites a method for producing only glucosamine by fermentation, lines 9-10 recite a method step of accumulating a product selected from glucosamine-6-phosphate and glucosamine. It is not clear if applicants intended to recite in the preamble of claim 61 that the method is directed toward producing glucosamine-6-phosphate or glucosamine. Claims 62-72 which depend from claim 61 are also rejected because they do not correct the defect of claim 61.

For examination purposes, claim 61 is assumed to be a method to produce glucosamine by fermentation and that the recovering step is directed only toward recovering and purifying the produced glucosamine.

In claim 61, lines 7-8, the phrase "said nucleic acid sequence a glucosamine-6-phosphate synthase" renders the claim vague and indefinite because it is not clear as to what nucleic acid applicant intends to be regulated through induction of a *lac* promoter. Amending the phrase to read "said nucleic acid sequence encoding a glucosamine-6-phosphate synthase" may overcome this ground of rejection.

Claim 68 is vague and indefinite because the claim recites amino acid modifications at specific positions is SEQ ID NO: 16 but does not recite any effect on the activity of the modified glucosamine-6-phosphate synthase. It is unclear if applicant intended that the recited amino acid modifications result in a glucosamine-6-phosphate synthase has reduced production inhibition, increased enzyme activity, or any other phenotypic effect.

Furthermore, claim 68 recites the limitation "wherein said at least one nucleic acid modification" in line 1. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 U.S.C. § 112, 1st Paragraph

6. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 61-64, 66-72 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 61 encompasses methods for making glucosamine by culturing any microorganism which is transformed with any nucleic acid encoding any glucosamine-6-phosphate synthase wherein said nucleic acid glucosamine-6-phosphate synthase is regulated through induction of a *lac* promoter.

The claim is a genus claim that is directed toward any glucosamine-6-phosphate synthase from any biological source and of any amino acid sequence and structure. The scope of the

claims includes many glucosamine-6-phosphate synthase enzymes from many biological sources with widely differing structural, chemical, and physical characteristics. Furthermore, the genus is highly variable because a significant number of structural differences between genus members is permitted.

The specification discloses a polynucleotide from *E.coli* encoding a wild-type *E.coli* glucosamine-6-phosphate synthase having the amino acid sequence of SEQ ID NO: 16; three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthase having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification); and a modified polynucleotide (SEQ ID NO: 30) encoding a mutant *E.coli* glucosamine-6-phosphate synthase having a single amino acid substitutions of Gly472Ser in SEQ ID NO: 16 resulting in a mutant *E.coli* glucosamine-6-phosphate synthases that has increased enzyme activity compared to wild-type glucosamine-6-phosphate synthase (see Example 9 of specification).

However, neither the specification nor the general knowledge of those skilled in the art provide evidence of any significant structure and amino acid sequence which would be expected to be common to all members of the genus, where the genus includes glucosamine-6-phosphate synthases from bacteria, plants, mammals, and viruses. Alignment of SEQ ID NO: 16 to amino acid sequences of glucosamine-6-phosphate synthases from human, virus, and yeast show low amino acid identities of 36%, 41%, and 40% to SEQ ID NO: 16, respectively (see attached alignments for Accession NP_002047, Accession BAD15299, and Accession EAL02907).

Thus, the disclosed *E.coli* glucosamine-6-phosphate synthase having the amino acid sequence of SEQ ID NO: 16 is not representative of the claimed genus since other members of the genus have amino acid sequences and structures that are different from the disclosed *E.coli* glucosamine-6-phosphate synthase.

In view of the above considerations, one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed by members of the genus. Accordingly, Applicant has failed to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicant was in possession of the claimed invention.

Claims 62-64, 66, 67, 69-72 which depend from claim 61 are also rejected because they do not correct the defect of claim 61.

Claim 66 fails to comply with the written description requirement for the following

additional reasons. Claim 66 encompasses methods for making glucosamine by culturing any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase comprising a genetic modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said nucleic acid molecule encoding any glucosamine-6-phosphate synthase, wherein said at least one nucleotide modification results in increased glucosamine-6-phosphate synthase activity.

The claim is a genus claim that is directed toward any glucosamine-6-phosphate synthase from any biological source and of any amino acid sequence and structure encoded by any nucleic acid molecule which comprises a genetic modification selected from the group consisting of deletion, insertion, and substitution of at least any one nucleotide of said nucleic acid molecule, said modification resulting in increased glucosamine-6-phosphate synthase activity. The scope of the claims includes many glucosamine-6-phosphate synthase enzymes from many biological sources with widely differing structural, chemical, and physical characteristics, and many nucleic acid molecules having any deletion, insertion, and substitution of at least any one nucleotide of said encoding nucleic acid molecules. Furthermore, the genus is highly variable because a significant number of structural differences between genus members is permitted.

The specification discloses three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification); and a modified polynucleotide (SEQ ID NO: 30) encoding a mutant *E.coli* glucosamine-6-phosphate synthase having a single amino acid substitution of G472S in SEQ ID NO: 16 resulting in a mutant *E.coli* glucosamine-6-phosphate synthases that has increased enzyme activity compared to wild-type glucosamine-6-phosphate synthase (see Example 9 of specification).

However, the specification fails to provide a written description of additional representative polynucleotides from additional biological sources having deletion, insertion, or substitution of at least any one nucleotide of any polynucleotide encoding any glucosamine-6-phosphate synthase, wherein said at least one nucleotide modification results in increased glucosamine-6-phosphate synthase activity as encompassed by the genus claim.

In view of the above considerations, one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed by members of the genus. Accordingly, Applicant has failed to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicant was in possession of the claimed invention.

Claim 67 fails to comply with the written description requirement for the following additional reasons. Claim 67 encompasses methods for making glucosamine by culturing any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase comprising any genetic modification which reduces product inhibition of said glucosamine-6-phosphate synthase.

The claim is a genus claim that is directed toward any glucosamine-6-phosphate synthase from any biological source and of any amino acid sequence and structure encoded by any nucleic acid molecule which comprises any genetic modification which reduces product inhibition of said glucosamine-6-phosphate synthase. The scope of the claims includes many glucosamine-6-phosphate synthase enzymes from many biological sources with widely differing structural, chemical, and physical characteristics, and many nucleic acid molecules having any genetic modification of any type which reduces product inhibition of said glucosamine-6-phosphate synthase. Furthermore, the genus is highly variable because a significant number of structural differences between genus members is permitted.

The specification discloses three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification).

However, the specification fails to provide a written description of additional representative polynucleotides from additional biological sources having any genetic modification of any type which reduces product inhibition of said glucosamine-6-phosphate synthase as encompassed by the genus claim.

In view of the above considerations, one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed by members of the genus. Accordingly, Applicant has failed to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicant was in possession of the claimed invention.

Claim 68 fails to comply with the written description requirement for the following additional reasons. Claim 68 encompasses methods for making glucosamine by culturing any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase, wherein said nucleic acid molecule has at least one nucleic acid modification resulting in any amino acid modification at specific amino acid positions of SEQ ID NO: 16.

The claim is a genus claim that is directed toward any glucosamine-6-phosphate synthase from any biological source and of any amino acid sequence and structure encoded by any nucleic

acid molecule having any nucleic acid modification that results in any amino acid modification at an amino acid position in SEQ ID NO: 16 selected from the group consisting of Ile(4), Ile(272), Ser(450), Ala(39), Arg(250), Gly(472), Leu(469), and combinations thereof.

The scope of the claims includes many glucosamine-6-phosphate synthase enzymes from many biological sources with widely differing structural, chemical, and physical characteristics, and many nucleic acid molecules having any type of nucleic acid modification which results in any amino acid modification (i.e., substitution, deletion, insertion, addition, and combinations thereof) at an amino acid position in SEQ ID NO: 16 selected from the group consisting of Ile(4), Ile(272), Ser(450), Ala(39), Arg(250), Gly(472), Leu(469), and combinations thereof. Furthermore, the genus is highly variable because a significant number of structural differences between genus members is permitted.

The specification discloses three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification). The specific single amino acid substitutions in SEQ ID NO: 16 are Ile4Thr, Ile272Thr, Ser45Pro, Ala39Thr, Arg250Cys, Gly472Ser, and Leu469Pro.

However, the specification fails to provide a written description of additional representative nucleic acid molecules from additional biological sources having any type of nucleic acid modification which results in any amino acid modification (i.e., substitution, deletion, insertion, addition, and combinations thereof) at an amino acid position in SEQ ID NO: 16 selected from the group consisting of Ile(4), Ile(272), Ser(450), Ala(39), Arg(250), Gly(472), Leu(469), and combinations thereof, as encompassed by the genus claim.

In view of the above considerations, one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed by members of the genus. Accordingly, Applicant has failed to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicant was in possession of the claimed invention.

Claims 70 and 71 fail to comply with the written description requirement for the following additional reasons. Claims 70 and 71 encompasses methods for making glucosamine by culturing the microorganism of claim 61 which further has at least one additional genetic modification in any gene encoding any of the proteins or enzymes recited in claims 70 and 71 which decreases the activity of the said proteins or enzymes.

The claim is a genus claim that is directed toward any gene from any biological source of

any nucleotide sequence and structure encoding any of the proteins or enzymes recited in claims 70 and 71, where the said any gene is modified by any genetic modification which decreases the activity of the said proteins or enzymes. The scope of the claims includes many genes from many biological sources with widely differing structural, chemical, and physical characteristics; and many genetic modifications in the nucleotide sequences of the genes encoding the recited proteins and enzymes (i.e., nucleotide substitution, deletion, insertion, addition, and combinations thereof) that decreases the activity of the recited proteins or enzymes of claims 70 and 71.

The specification discloses three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification). The specific single amino acid substitutions in SEQ ID NO: 16 are Ile4Thr, Ile272Thr, Ser45Pro, Ala39Thr, Arg250Cys, Gly472Ser, and Leu469Pro.

However, the specification fails to provide a written description of any genetic modification to any of the genes encoding any of the proteins or enzymes recited in claims 70 and 71 which decreases the activity of the said proteins or enzymes, as encompassed by the genus claim. Neither the specification nor the general knowledge of those skilled in the art provide evidence of any genetic modification, other than deletion of the recited genes in the microorganism, which would decrease the activity of the proteins or enzymes recited in claims 70 and 71.

In view of the above considerations, one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed by members of the genus. Accordingly, Applicant has failed to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicant was in possession of the claimed invention.

8. Claims 66-68, 70, and 71 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for method for making glucosamine by culturing a microorganism which is transformed with a nucleic acid molecule comprising a nucleic acid sequence encoding a glucosamine-6-phosphate synthase of SEQ ID NO: 16, or transformed with the polynucleotide of SEQ ID NOs: 18, 21, and 24; does not reasonably provide enablement for any other embodiment. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with this claim.

Factors to be considered in determining whether undue experimentation is required, are summarized In re Wands [858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)]. The Wands factors are: (a) the quantity of experimentation necessary, (b) the amount of direction or guidance presented, (c) the presence or absence of working example, (d) the nature of the invention, (e) the state of the prior art, (f) the relative skill of those in the art, (g) the predictability or unpredictability of the art, and (h) the breadth of the claim.

The nature and breadth of claim 66 encompass any method for making glucosamine by culturing any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase from any biological source comprising a genetic modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said nucleic acid molecule encoding any glucosamine-6-phosphate synthase, wherein said at least one nucleotide modification results in increased glucosamine-6-phosphate synthase activity.

In order to meet the enablement requirement, one skilled in the art must be able to make any nucleic acid molecule encoding any glucosamine-6-phosphate synthase comprising a genetic modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said nucleic acid molecule encoding any glucosamine-6-phosphate synthase resulting in increased glucosamine-6-phosphate synthase activity, without undue experimentation using the specification coupled with information known in the art. However, neither the specification nor the general knowledge of those skilled in the art provide guidance or prediction on making the invention of claim 66 without undue experimentation.

The specification discloses three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification); and a modified polynucleotide (SEQ ID NO: 30) encoding a mutant *E.coli* glucosamine-6-phosphate synthase having a single amino acid substitution of G472S in SEQ ID NO: 16 resulting in a mutant *E.coli* glucosamine-6-phosphate synthases that has increased enzyme activity compared to wild-type glucosamine-6-phosphate synthase (see Example 9 of specification).

However, the specification does not provide guidance or prediction regarding the specific structural and catalytic amino acids residues in any glucosamine-6-phosphate synthase from any biological source that are essential for enzyme activity which cannot be altered and specific amino acid residues that can be changed which results in increased glucosamine-6-phosphate synthase activity.

The specification only provides working examples for three modified polynucleotides

(SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition or increased enzyme activity compared to wild-type *E.coli* glucosamine-6-phosphate synthase.

The general knowledge of those skilled in the art does not provide any guidance or prediction regarding the specific structural and catalytic amino acids residues in any glucosamine-6-phosphate synthase from any biological source that are essential for enzyme activity which cannot be altered and specific amino acid residues that can be changed which results in increased glucosamine-6-phosphate synthase activity. The prior art as exemplified by Broun et al. (Science. 1998 Nov 13;282(5392):1315-7) teach that minor modifications to a protein sequence can completely alter the function of a protein. Broun et al. show that as few as four amino acid substitutions in a polypeptide consisting of 380 amino acid residues changes the enzymatic activity of the polypeptide from a desaturase to a hydroxylase (seen entire publication, especially the abstract and pp. 1316-1317).

Since neither the specification nor information known in the art provide guidance or prediction for the specific structural and catalytic amino acids residues in any glucosamine-6-phosphate synthase from any biological source that are essential for enzyme activity which cannot be altered and specific amino acid residues that can be changed which results in increased glucosamine-6-phosphate synthase activity, one must perform an enormous amount of trial and error experimentation to determine which amino acid residues can be changed which results in increased glucosamine-6-phosphate synthase activity and then construct a modified nucleic acid molecule having the corresponding nucleotide deletion, insertion, and substitution which will encode the modified glucosamine-6-phosphate synthase that has increased enzyme activity.

Such trial and error experimentation is well outside the realm of routine experimentation and entails searching for any glucosamine-6-phosphate synthase from any biological source; isolating the polynucleotide encoding the glucosamine-6-phosphate synthase; performing any deletion, insertion, and substitution of any nucleotides in the polynucleotide encoding the glucosamine-6-phosphate synthase; and searching and screening for modified polynucleotides that encode glucosamine-6-phosphate synthase enzymes that have increased enzyme activity. Teaching regarding screening and searching for modified polynucleotides that have nucleotide deletion, insertion, and substitution and encode glucosamine-6-phosphate synthase with increased enzyme activity using enzyme assays stated in the specification is not guidance for making the claimed invention.

The Examiner finds that one skilled in the art would require additional guidance, such as information regarding the amino acid residues which can be changed without inactivating enzyme activity and amino acid residues that can be changed which results in increased enzyme activity.

Without such a guidance, the amount of experimentation left to those skilled in the art to make the invention of claim 66 is undue and well outside of routine experimentation.

The nature and breadth of claim 67 encompass any method for making glucosamine by culturing any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase comprising any genetic modification which reduces product inhibition of said glucosamine-6-phosphate synthase.

In order to meet the enablement requirement, one skilled in the art must be able to make any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase comprising any genetic modification which reduces product inhibition of any glucosamine-6-phosphate synthase, without undue experimentation using the specification coupled with information known in the art. However, neither the specification nor the general knowledge of those skilled in the art provide guidance or prediction on making the invention of claim 67 without undue experimentation.

The specification discloses three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification).

However, the specification does not provide guidance or prediction regarding the specific structural and catalytic amino acids residues in any glucosamine-6-phosphate synthase from any biological source that are essential for enzyme activity which cannot be altered and specific amino acid residues that can be changed which reduces product inhibition of said glucosamine-6-phosphate synthase activity. The specification only provides working examples for three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase.

The general knowledge of those skilled in the art does not provide any guidance or prediction regarding the specific structural and catalytic amino acids residues in any glucosamine-6-phosphate synthase from any biological source that are essential for enzyme activity which cannot be altered and specific amino acid residues that can be changed which reduces product inhibition of any glucosamine-6-phosphate synthase.

Since neither the specification nor information known in the art provide guidance or prediction for the specific structural and catalytic amino acids residues in any glucosamine-6-phosphate synthase from any biological source that are essential for enzyme activity which cannot

be altered and specific amino acid residues that can be changed which reduces product inhibition of any glucosamine-6-phosphate synthase, one must perform an enormous amount of trial and error experimentation to determine which amino acid residues can be changed which reduces product inhibition and then construct a modified nucleic acid molecule which will encode the modified glucosamine-6-phosphate synthase that has reduced product inhibition.

Such trial and error experimentation is well outside the realm of routine experimentation and entails searching for any glucosamine-6-phosphate synthase from any biological source; isolating the polynucleotide encoding the glucosamine-6-phosphate synthase; performing any modification (deletion, insertion, substitution, and combinations thereof) of any nucleotide in the polynucleotide encoding the glucosamine-6-phosphate synthase; and searching and screening for modified polynucleotides that encode glucosamine-6-phosphate synthase enzymes that have reduced production inhibition. Teaching regarding screening and searching for modified polynucleotides that encode glucosamine-6-phosphate synthase with reduced production inhibition using enzyme assays stated in the specification is not guidance for making the claimed invention.

The Examiner finds that one skilled in the art would require additional guidance, such as information regarding the amino acid residues which can be changed without inactivating enzyme activity and amino acid residues that can be changed which reduces glucosamine-6-phosphate synthase product inhibition. Without such a guidance, the amount of experimentation left to those skilled in the art to make the invention of claim 67 is undue and well outside of routine experimentation.

The nature and breadth of claim 68 encompass any method for making glucosamine by culturing any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase, wherein said nucleic acid molecule has at least one nucleic acid modification resulting in any amino acid modification at specific amino acid positions of SEQ ID NO: 16 selected from the group consisting of Ile(4), Ile(272), ser(450), Ala(39), Arg(250), Gly(472), Leu(469), and combinations thereof. For examination purposes, claim 68 is assumed to depend from claim 67 and have the limitation that the glucosamine-6-phosphate synthase has reduced product inhibition.

In order to meet the enablement requirement, one skilled in the art must be able to make any microorganism which is transformed with any nucleic acid molecule encoding any glucosamine-6-phosphate synthase having reduced product inhibition, wherein said nucleic acid molecule has at least one nucleic acid modification resulting in any amino acid modification at specific amino acid positions of SEQ ID NO: 16 selected from the group consisting of Ile(4), Ile(272), ser(450), Ala(39), Arg(250), Gly(472), Leu(469), and combinations thereof, without

undue experimentation using the specification coupled with information known in the art. However, neither the specification nor the general knowledge of those skilled in the art provide guidance or prediction on making the invention of claim 68 without undue experimentation.

The specification discloses three modified polynucleotides (SEQ ID NOs: 18, 21, and 24) encoding mutant *E.coli* glucosamine-6-phosphate synthases having single amino acid substitutions at specific positions in SEQ ID NO: 16 resulting in mutant *E.coli* glucosamine-6-phosphate synthases that have reduced product inhibition compared to wild-type *E.coli* glucosamine-6-phosphate synthase (see Table 5 on p.89 of specification). The specific single amino acid substitutions in SEQ ID NO: 16 are Ile4Thr, Ile272Thr, Ser45Pro, Ala39Thr, Arg250Cys, Gly472Ser, and Leu469Pro.

However, the specification does not provide guidance or prediction regarding any nucleic acid modification that results in any amino acid modification in SEQ ID NO: 16 at the recited positions that would yield a glucosamine-6-phosphate synthase that has reduced production inhibition, other than the single amino acid substitutions in SEQ ID NO: 16 of Ile4Thr, Ile272Thr, Ser45Pro, Ala39Thr, Arg250Cys, Gly472Ser, and Leu469Pro.

Hence, one must perform an enormous amount of trial and error experimentation to determine the specific "amino acid modification" to perform which reduces product inhibition and then construct a modified nucleic acid molecule which will encode the modified glucosamine-6-phosphate synthase that has reduced product inhibition. Such trial and error experimentation is well outside the realm of routine experimentation and entails searching and screening for any type of amino acid modification (substitution, deletion, insertion, addition, and combinations thereof with other amino acids) to the amino acids at the recited positions of SEQ ID NO: 16 and then constructing the corresponding polynucleotide that encodes the glucosamine-6-phosphate synthase that has reduced production inhibition. Teaching regarding screening and searching for modified polynucleotides that encode glucosamine-6-phosphate synthase with reduced production inhibition using enzyme assays stated in the specification is not guidance for making the claimed invention.

The Examiner finds that one skilled in the art would require additional guidance, such as information regarding the specific amino acids that can be substituted into the recited positions of SEQ ID NO: 16 which would result in a glucosamine-6-phosphate synthase that has reduced production inhibition. Without such a guidance, the amount of experimentation left to those skilled in the art to make the invention of claim 68 is undue and well outside of routine experimentation.

The nature and breadth of claims 70 and 71 encompass any method for making glucosamine by culturing the microorganism of claim 61 which further has at least one additional

genetic modification in any gene encoding any of the proteins or enzymes recited in claims 70 and 71 which decreases the activity of the said proteins or enzymes.

In order to meet the enablement requirement, one skilled in the art must be able to make the microorganism of claim 61 which further has any additional genetic modification in any gene encoding any of the proteins or enzymes recited in claims 70 and 71 which decreases the activity of the said proteins or enzymes, without undue experimentation using the specification coupled with information known in the art. However, neither the specification nor the general knowledge of those skilled in the art provide guidance or prediction on making the invention of claims 70 and 71 without undue experimentation.

The specification does not provide guidance or prediction regarding any type of genetic modification to any of the genes encoding any of the proteins or enzymes recited in claim 70 and 71 that would decrease the activity of said proteins or enzymes. Hence, one must perform an enormous amount of trial and error experimentation to determine the specific genetic modification to any of the genes which would decrease the activity of the proteins or enzymes of claims 70 and 71. Such trial and error experimentation is well outside the realm of routine experimentation and entails searching and screening for any type genetic modification (nucleotide substitution, deletion, insertion, addition, and combinations thereof) to any of the genes which would decrease the activity of the proteins or enzymes of claims 70 and 71. Teaching regarding screening and searching for modified genes that encode modified proteins or enzymes of claims 70 or 71 which have decreased activity is not guidance for making the claimed invention.

The Examiner finds that one skilled in the art would require additional guidance, such as information regarding the specific genetic modification to the proteins or enzymes of claims 70 and 71 which would decrease the activity of said proteins or enzymes. Without such a guidance, the amount of experimentation left to those skilled in the art to make the invention of claims 70 and 71 is undue and well outside of routine experimentation.

Claim Rejections - 35 U.S.C. § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claims 61, 63, 64, 72 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dutak-Malen et al. (Biochimie. 1988 Feb;70(2):287-90) in view of Szymona et al. (Annales Universitatis Mariae Curie-Sklodowska, Sectio D: Medicina (1980), Volume Date 1979, 35, pp. 1-6) and Woodall et al. (J. Bacteriol. 1993 May; 175(9):2770-8).

Dutak-Malen et al. teach the following:

(1) plasmid pGM10 was constructed containing the *E.coli* glms gene which encodes the *E.coli* glucosamine synthetase (glucosamine-6-phosphate synthase) under control of the *lac* promoter; and transformation of *E.coli* cells with said plasmid pGM10 to make *E.coli* NK7356 which harbors plasmid pGM10 (see entire publication, especially the **Results and Discussion** section beginning on p. 288 to p. 290)

(2) *E.coli* NK7356 was cultured in LB medium with subsequent addition of lactose to induce overexpression of glucosamine-6-phosphate synthase (see ***Enzyme assay and purification*** section on p. 288) ; glucosamine-6-phosphate overproduction in *E.coli* NK7356 was confirmed when enzyme assay which measures the amount of glucosamine-6-phosphate were 2-fold higher compared to an *E.coli* strain that has a plasmid containing the glms gene in the other orientation (see p. 289, left column, lines 20-31)

(3) *E.coli* NK7356 was harvested by centrifugation and a crude extract was made through alumina disruption of cells (see p. 288, right column, lines 2-7).

Claims 61, 63, 64, 72 differ from the teachings of Dutak-Malen et al. in that there is no recovering and purifying of the produced glucosamine-6-phosphate and the *lacUV5* promoter is used.

Szymona et al. teach process steps for isolation of glucosamine-6-phosphate as barium salt from a reaction mixture (see entire publication, especially SCHEME 1 on p. 4).

Wood all et al. teach a plasmid containing the chromosomal fimA gene under the control of the lacUV5 promoter, transformation of the said plasmid into E.coli, induction of type 1 pilation in E.coli by addition of isopropyl-beta-D-thiogalactopyranoside (IPTG) (see entire publication, especially p. 2270, right column, last paragraph to p. 2771, left column; Figure 1 on p. 2772; and p. 2773, right column, line 6 to end of p.2777).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Dutak-Malen et al. such that the glms gene encoding

glucosamine -6-phosphate synthase is linked and placed under the control of the lacUV5 promoter as taught by Woodall et al.; and the crude extract of *E.coli* NK7356 is then subject to precipitation with barium salt for the isolation glucosamine -6-phosphate as taught by Szymona et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do this. in order to create a controllable and beneficial method for producing glucosamine, where expression is under the control of addition of IPTG

No patentable weight is given to the preamble of the process claims since it merely recites the purpose of these process claims. Because the process steps of the modified process of Dutak-Malen et al. stated above are the same as the process steps of claims 61, 63, 64, 72; then the modified process of Dutak-Malen et al. stated above would inherently produce glucosamine or glucosamine-6-phosphate.

11. Claim 62 is rejected under 35 U.S.C. 103(a) as being unpatentable over Dutak-Malen et al. in view of Szymona et al. and Woodall et al. as applied to claims 61, 63, 64, 72 above, and further in view of Zhang et al. (J Bacteriol. 1996 Jan;178(2):490-5), Copeland et al. (Arch Biochem Biophys. 1995 Oct 20;323(1):79-86), and Studier et al. (J Mol Biol. 1986 May 5;189(1):113-30).

Zhang et al. teach that the msdA gene was overexpressed in *Escherichia coli* under the control of the T7 promoter (see entire publication)

Copeland et al. teach a cDNA for human dihydroorotate dehydrogenase (DHODase) placed under control of the inducible T7 lac promoter (see entire publication).

Studier et al. teach a nucleic acid encoding a T7 RNA polymerase to direct high-level expression of cloned genes (see entire publication).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to further modify the modified teachings of Dutak-Malen et al. stated above such that the nucleic acid encoding glucosamine-6-phosphate synthase is linked to the T7 promoter taught by Zhang et al. or the T7-lac promoter taught by Copeland et al. for the purposes of a controlled and/or inducible expression of the glucosamine-6-phosphate synthase. It would have been obvious to one of ordinary skill in the art at the time the invention was made to also transform the *E.coli* with a nucleic acid encoding a T7 RNA polymerase for the purpose of directed high-level expression of the glucosamine-6-phosphate synthase.

12. Claim 69 is rejected under 35 U.S.C. 103(a) as being unpatentable over Dutak-Malen et al. in view of Szymona et al. and Woodall et al. as applied to claims 61, 63, 64, 72 above, and further in view of Balbas et al. (Gene. 1996 Jun 12;172(1):65-9).

Balbas et al. teach pBRINT plasmids that integrate into *E.coli* chromosome, process for using said pBRINT plasmids for integrating cloned DNA into genome of said microorganism, and the advantage of stability and lack of undesired copy number effects compared to replicative plasmid vectors (see entire publication).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to further modify the modified teachings of Dutak-Malen et al. stated above such that the nucleic acid encoding glucosamine-6-phosphate synthase is cloned in the pBRINT plasmids which is then integrated into the *E.coli* chromosome, where such integration has the advantage of being stable and lack of undesired copy number effects as taught by Balbas et al..

Conclusion

13. No claims are allowed.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christian L Fronda whose telephone number is (571)272-0929. The examiner can normally be reached Monday-Friday between 9:00AM - 5:00PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura N Achutamurthy can be reached on (571)272-0928. The fax phone number for the organization where this application or proceeding is assigned is (571)273-8300.

15. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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